FIG.1

[SEQ. ID NO: 3] X-C-C-T-T-G-A-G-A-T-T-T-C-C-C-T-C

G-G-A-A-C-T-C-T-A-A-A-G-G-G-A-G-X
3' [SEQ. ID NO: 4]



X-C-C-T-T-G-A-G-A-T-T-T-C-C-C-T-C G-G-A-A-C-T-C-T-A-A-G-G-G-A-G-X

FIG.2

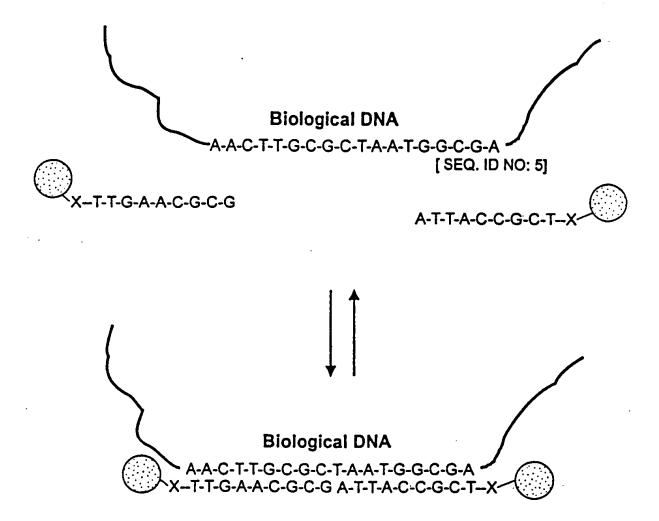
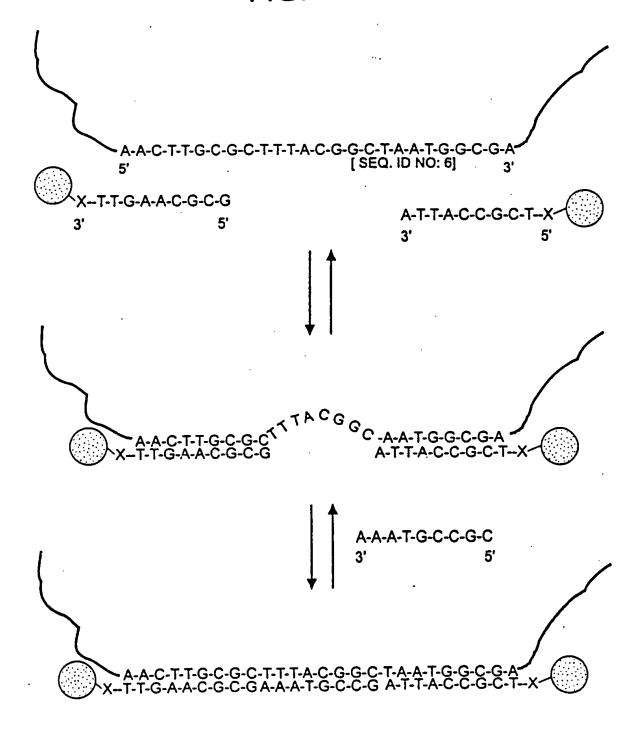


FIG.3



Linking oligonucleotide A-T-G-G-C-A-A-C-T-A-T-A-C-G-C-G-C-T-A-G

A-T-A-T-G-C-G-C-G-C-T-C-T-C-A-G-C-A-A-A

id No: 2]

3'
5'
6' [SEQ. ID NO:1] [SEQ. ID NO: 2]

X-T-A-C-C-G-T-T-G

A-G-T-C-G-T-T-X

Colloids

Heat Mix below Tm

Aggregate

A-T-G-G-C-A-A-C-T-A-T-A-C-G-C-G-C-T-A-G A-G-T-C-G-T-T-T-X X-T-A-C-C-G-T-T-G A-T-A-T-G-C-G-C-G-A-T-C-T-C-A-G-C-A-A-A 3' 5' Stand below Tm Heat

Precipitate (formed by further cross-linking)

FIG.5

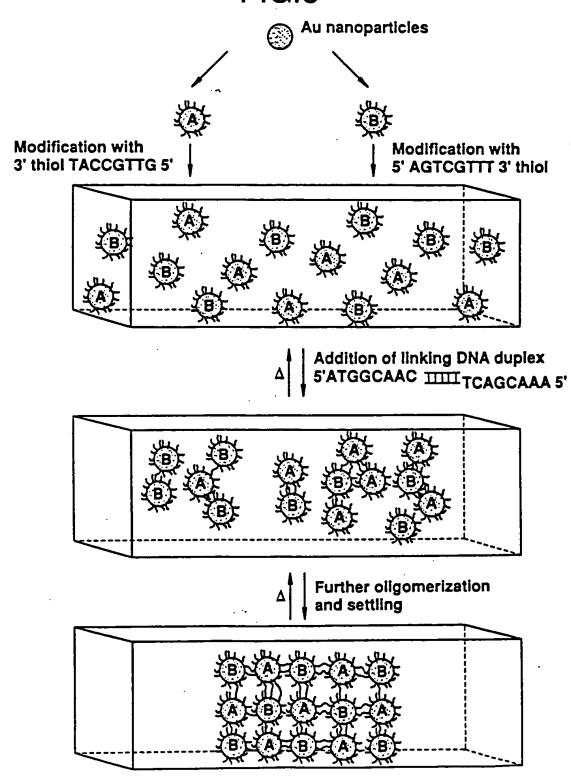
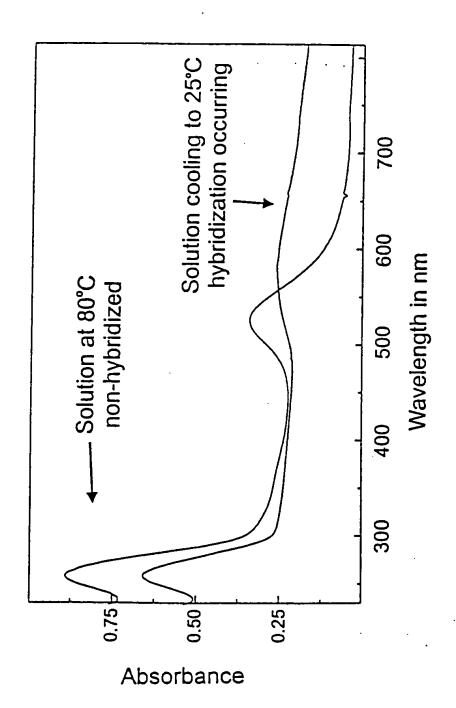




FIG.6A FIG.6B FIG.6C





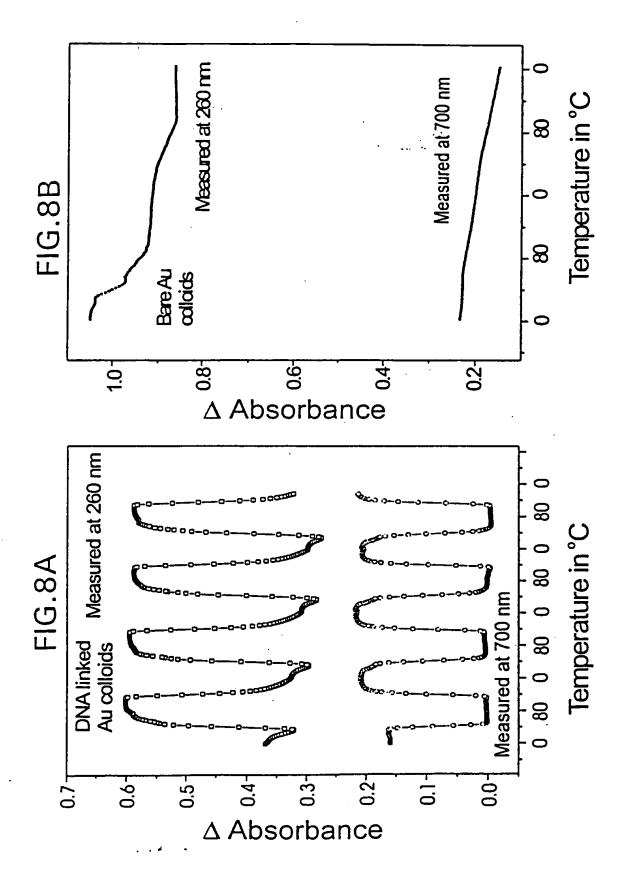




FIG.9A

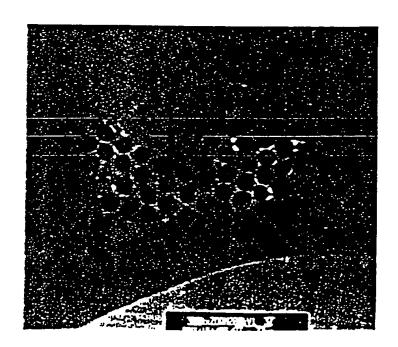
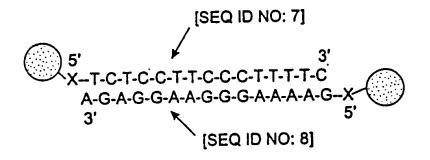
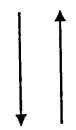


FIG.9B

FIG.10



3' T-C-T-C-C-T-T-C-C-C-T-T-T-C 5' [SEQ ID NO: 9]



5' 3' X--T-C-T-C-C-T-T-C-C-C-T-T-T-C A-G-A-G-G-A-A-G-G-A-A-A-A-G-X T-C-T-C-C-T-T-C-C-C-T-T-T-C 5'

FIG.11

[SEQ. ID NO: 10]

S-A-T-G-G-C-A-A-C-T-A-C-G-C-G-C-T-A-G-A-G-T-C-G-T-T-T

T-A-C-C-G-T-T-G-A-T-A-T-G-C-G-C-G-A-T-C-T-C-A-G-C-A-A-S-7 5' 3' [SEQ. ID NO: 11]



FIG.12A Complementary Target [SEQ. ID NO: 14] SEQ. ID NO:12 3' T-C-G-T-A-C-C-A-G-C-T-A-T-C-C T-T-T-G-C-T-G-A-G-A-T-C-G-C-G 5' A-G-C-A-T-G-G-T-C-G-A-T-A-G-G-A-A-A-C-G-A-C-T-C-T-A-G-C-G-C FIG.12B [SEQ. ID NO:13] Probes without Target 3' T-C-G-T-A-C-C-A-G-C-T-A-T-C-C T-T-T-G-C-T-G-A-G-A-T-C-G-C-G FIG.12C Half Complementary Target 3' T-C-G-T-A-C-C-A-G-C-T-A-T-C-C T-T-T-G-C-T-G-A-G-A-T-C-G-C-G 5' A-G-C-A-T-G-G-T-C-G-A-T-A-G-G-A[T-G-G-C]A[A-C-T-A-T-A]C-G-C [SEQ. ID NO: 15] FIG. 12D Target - 6 bp 3' T-C-G-T-A-C-C-A-G-C-T-A-T-C-C T-T-T-G-C-T-G-A-G-A-T-C-G-C-G 5' G-T-C-G-A-T-A-G-G-A-A-A-C-G-A-C-T-C-T-A-G-C-G-C [SEQ. ID NO:16] **FIG. 12E** One bp Mismatch 3' T-C-G-T-A-C-C-A-G-C-T-A-T-C-C T-T-T-G-C-T-G-A-G-A-T-C-G-C-G 5' A-G-C-A-T-G-G-T-TG-A-T-A-G-G-A-A-A-C-G-A-C-T-C-T-A-G-C-G-C [SEQ. ID NO: 17] FIG.12F Two bp Mismatch 3' T-C-G-T-A-C-C-A-G-C-T-A-T-C-C T-T-T-G-C-T-G-A-G-A-T-C-G-C-G 5' A-G-C-A-T-G①T①G-A-T-A-G-G-A-A-A-C-G-A-C-T-C-T-A-G-C-G-C ·[SEQ. ID NO:18]

FIG.13A

transparent substrate

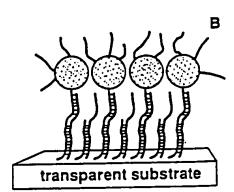
Modified DNA chemisorbed onto solid substrate

Analyte DNA hybridized onto substrate

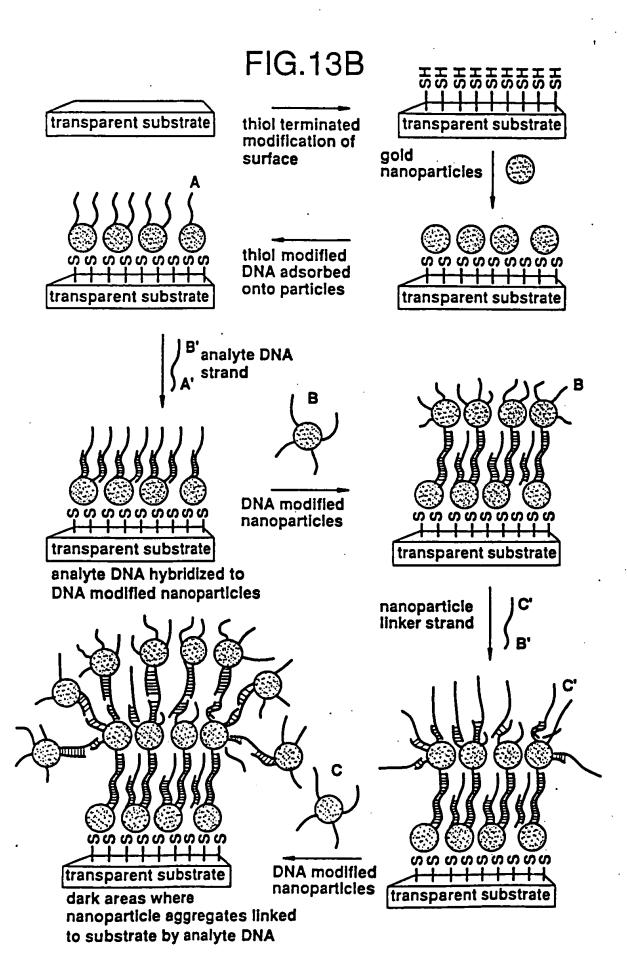
DNA modified colloids

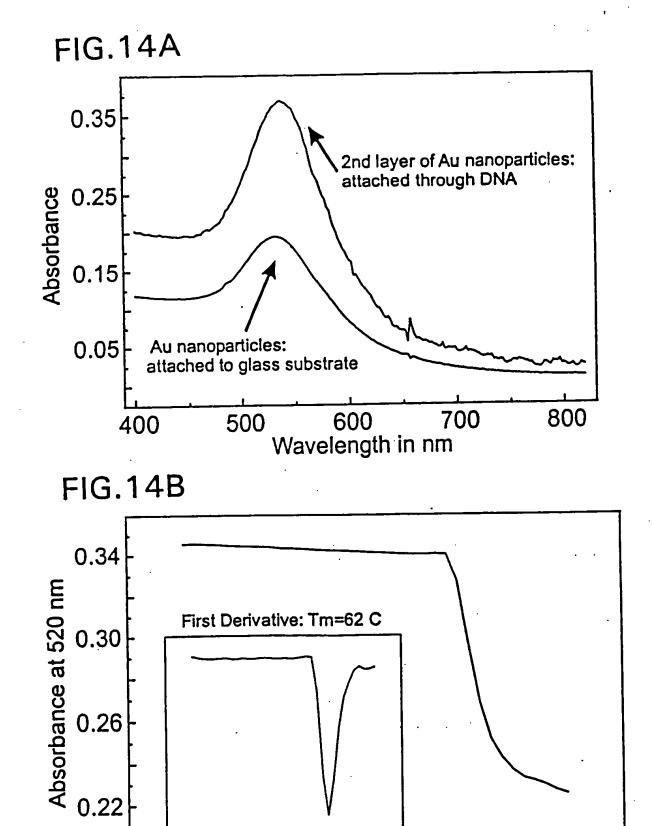
transparent substrate

Dark areas where nanoparticle aggregates are linked to substrate surface by analyte DNA



DNA modified colloids hybridized to bound analyte DNA





Temperature in °C

FIG15A Probes with No Target SEQ ID NO:19
Probes with No Target SEQ ID NO:19 SEQ ID NO:20
S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S
FIG15B
Half-Complementary Target
3 SEQ ID NO:21
5' TAC-GAG-TTG-AGA-GAG-TGC-CCA-CAT 3'
S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S
FIG15C
Complementary Target Tm=53.5°C
4 SEQ ID NO:22
5' TAC-GAG-TTG-AGA-ATC-CTG-AAT-GCG 3' S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S
1 2
FIG15D
ONE Base-Pair Mismatch at Probe Head Tm=50.4°C
5 SEQ ID NO:23
5' TAC-GAG-TTG-AGA-ATC-CTG-AAT-GC <u>T</u> 3'
S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S
FIG15E Tm=46.2°C
ONE Base-Pair Mismatch at Probe Tail
6 SEQ ID NO:24
5' TAC-GAG-TTG-AGA-CTC-CTG-AAT-GCG 3'
S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S 1 2
FIG15F Tm=51.6°C
ONE Base Deletion 7 SEQ ID NO:25
5' TAC-GAG-TTG-AGA-ATC-CTG-AAT-GC□ 3'
S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S 1 2
-
FIG15G ONE Base Pair Insertion Tm=50.2°C
ONE Base-Pair Insertion 8 SEQ ID NO:26
5' TAC-GAG-TTG-AGA-CAT-CCT-GAA-TGC-G 3'
S-ATG-CTC-AAC-TCT TA-GGA-CTT-ACG-C-S

FIG. 16A 24 Base Template

48 Base Template with Complementary 24 Base Filler FIG. 16B

5' TAC-GAG-TTG-AGA-CCG-TTA-AGA-CGA-GGC-AAT-CAT-GCA-ATC-CTG-AAT-GCG 3' S-ATG-CTC-AAC-TCT GGC-AAT-TCT-GCT-CCG-TTA-GTA-CGT TAG-GAC-TTA-CGC-S

72 Base Template with Complementary 48 Base Filler FIG. 16C

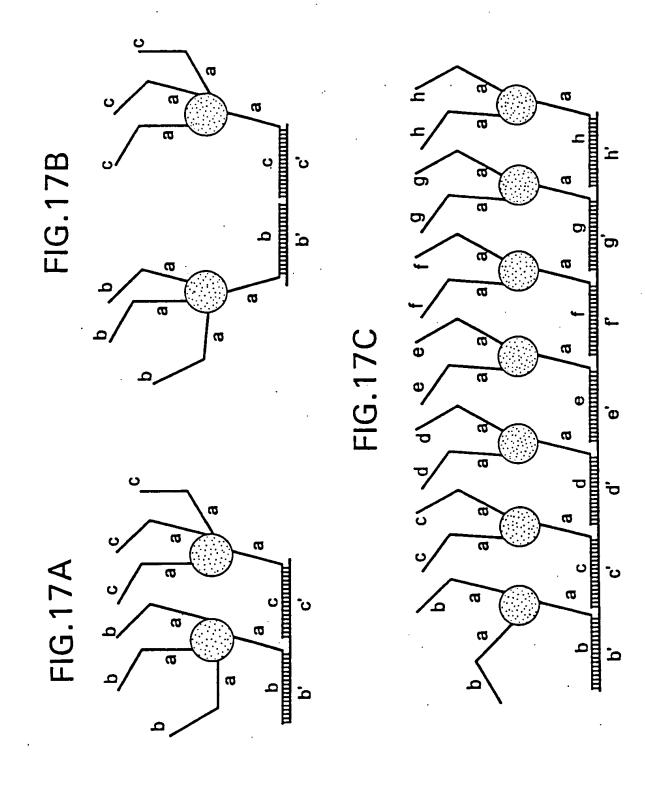
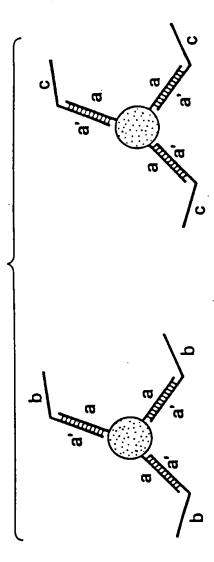


FIG.17D



A THE A THE

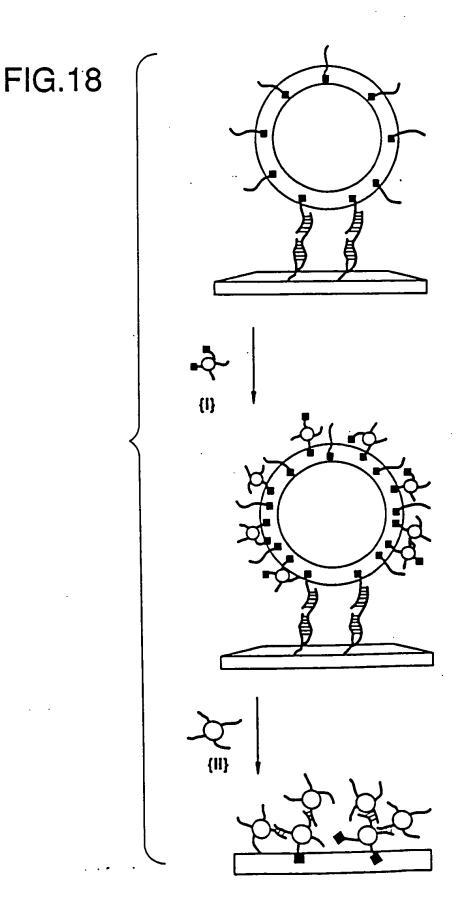
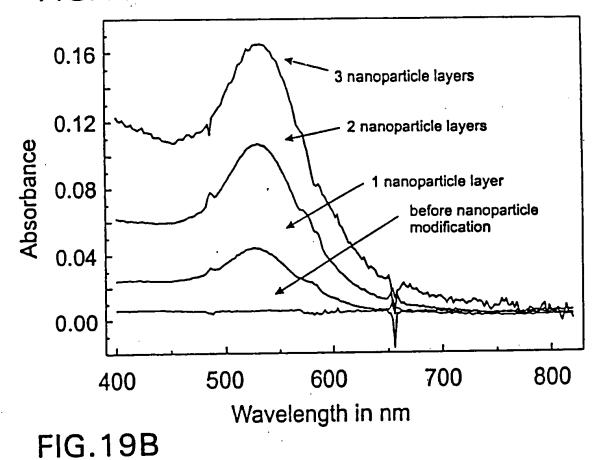


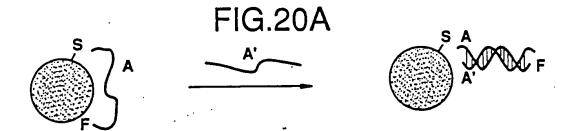
FIG.19A

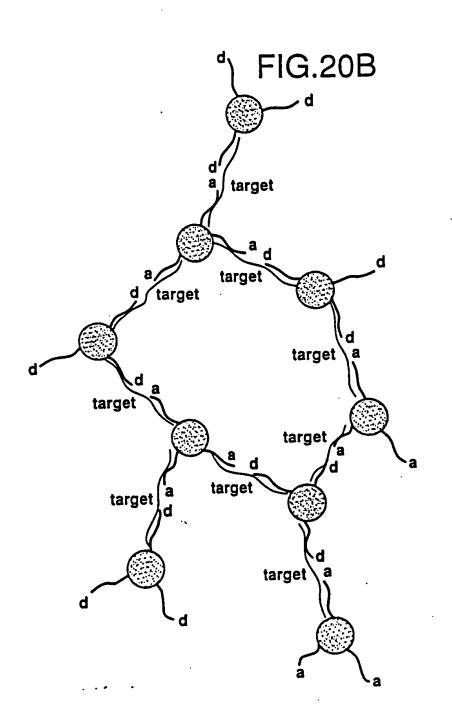


O.24 First Derivative: Tm=55°C

O.20

Temperature in °C





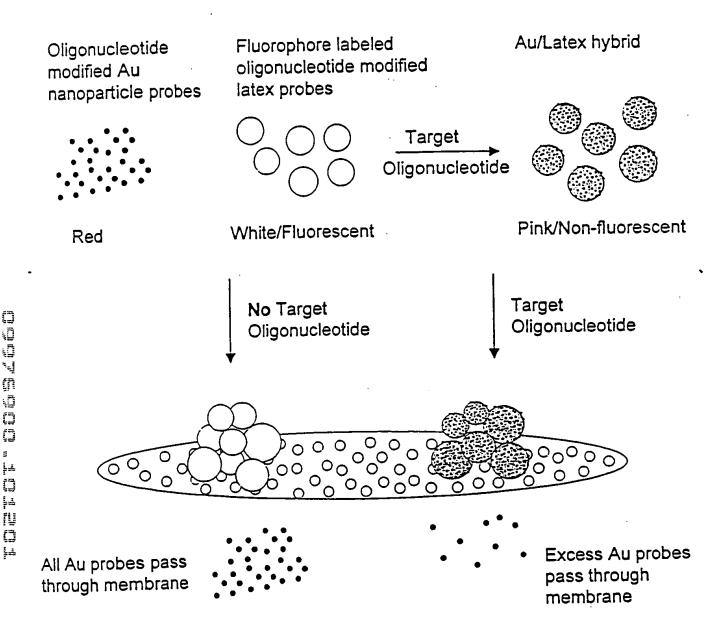
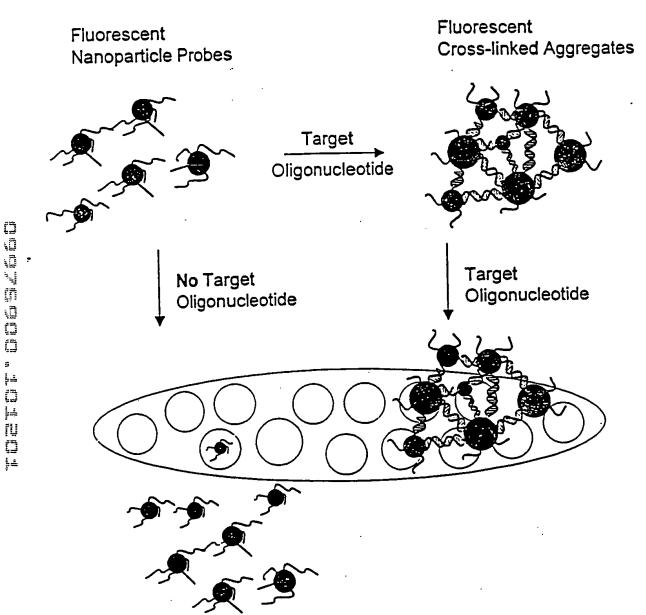


FIGURE 21



The fluorescent nanoparticle probes pass through the membrane

The fluorescent cross-linked aggregates are retained by the membrane

Anthrax PCR Product

5'G GCG GAT GAG TCA GTA GTT AAG GAG GCT CAT AGA GAA GTA ATT AAT 3'C CGC CTA CTC AGT CAT CAA TTC CTC CGA GTA TCT CTT CAT TAA TTA

TCG TCA ACA GAG GGA TTA TTG TTA AAT ATT GAT AAG GAT ATA AGA AAA AGC AGT TGT CTC CCT AAT AAC AAT TTA TAA CTA TTC CTA TAT TCT TTT

ATA TTA TCC AGG GTT ATA TTG TAG AAA TTG AAG ATA CTG AAG GGC TT 3'
TAT AAT AGG TCC CAA TAT AAC ATC TTT AAC TTC TAT GAC TTC CCG AA 5'

141 mer Anthrex PCR product [SEQ 10 NO:36]

3' CTC CCT AAT AAC AAT-

[SEQ ID NO:31]

3' TTA TAA CTA TTC CTA

[SEP ID NO: 38]

Oligonucleotide-Nanoparticle Probes

Blocker Oligonucleotides

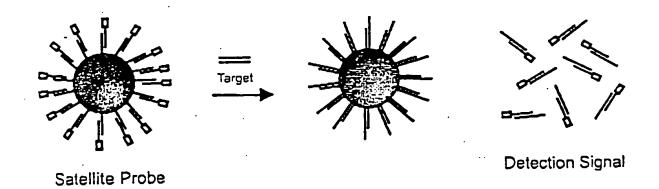
3' C CGC CTA CTC AGT CAT CAA TTC CTC CGA GT [SEQ 15 NO: 39]

3' A TCT CTT CAT TAA TTA AGC AGT TGT [SEQ 15 NO: 40]

3' TAT TCT TTT TAT AAT AGG TCC CAA TAT [SEQ 15 NO: 41]

3' AAC ATC TTT AAC TTC TAT GAC TTC CCG AA [SEQ 16 ND: 42]

FIGURE 23



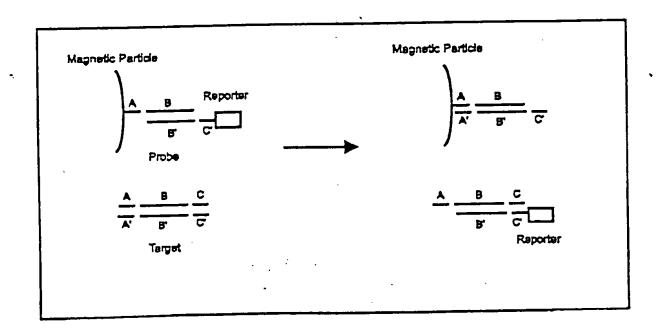
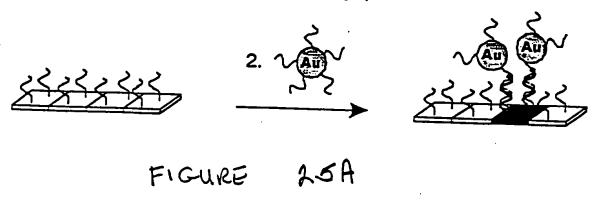
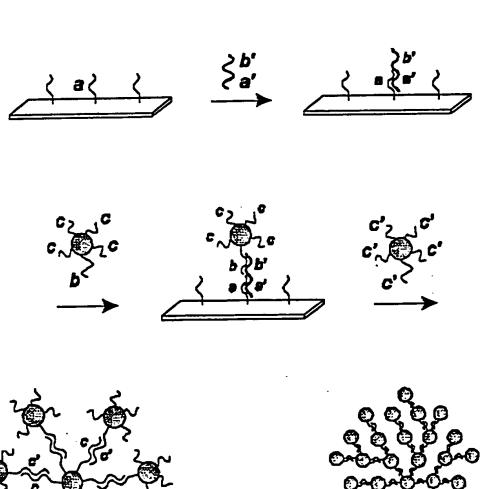


FIGURE 24

1. **(**target)





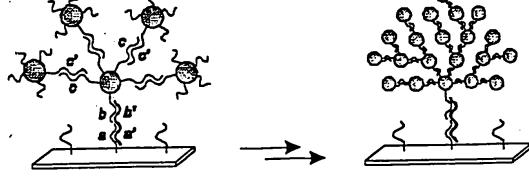


FIGURE 25 B

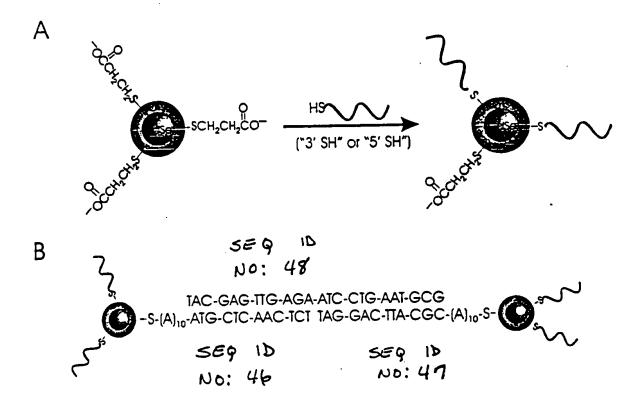
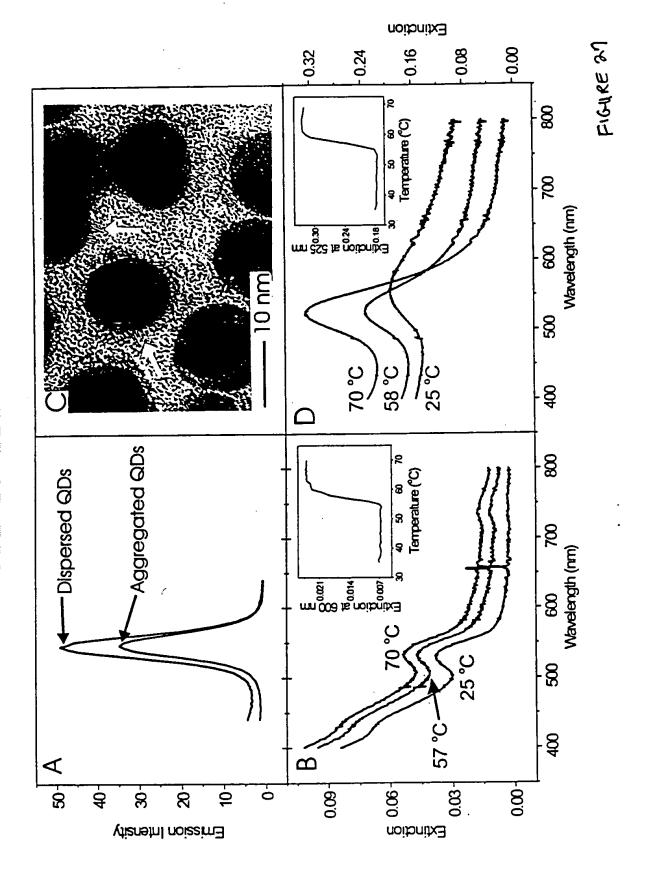


FIGURE 26

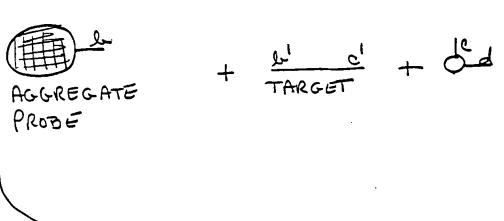


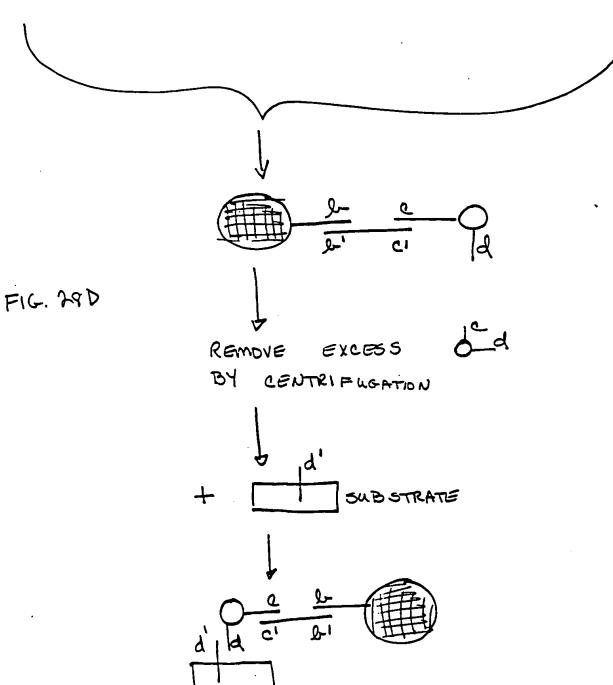
$$\frac{a + \frac{a'}{a'} \frac{a'}{a'}}{|a'|} \longrightarrow ORE$$

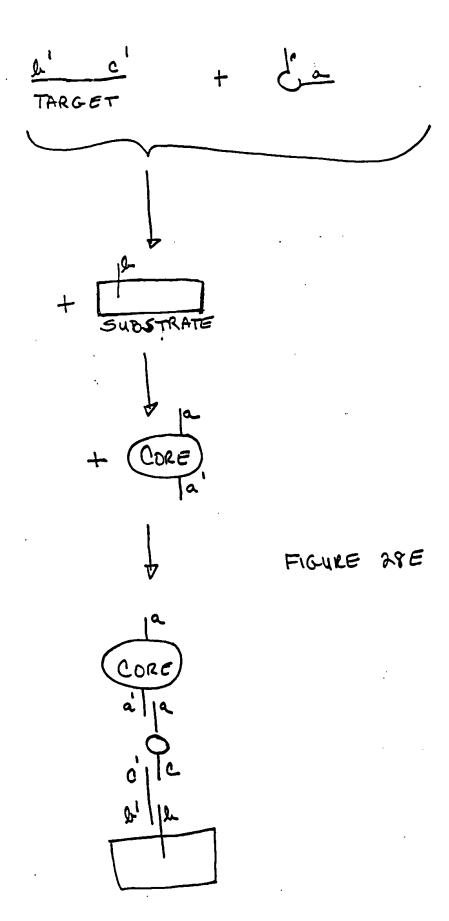
FIGURE Z8A

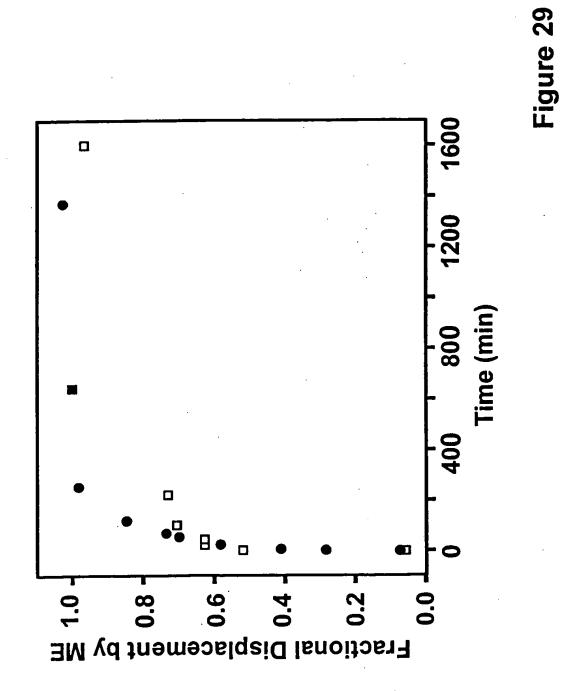
FIGURE AS B

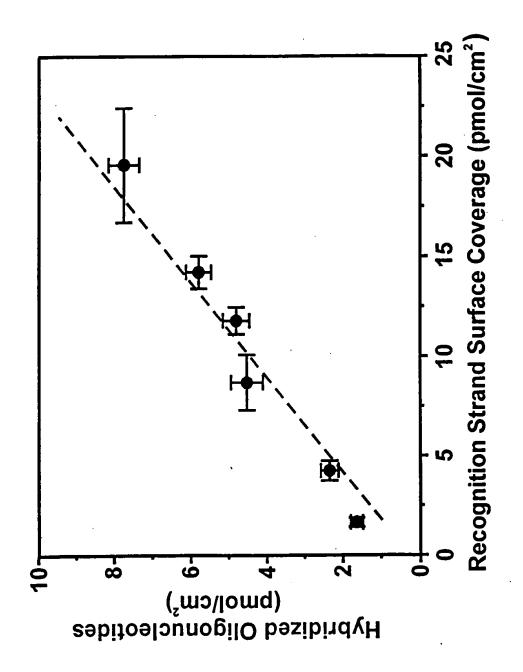
FIG. 28C

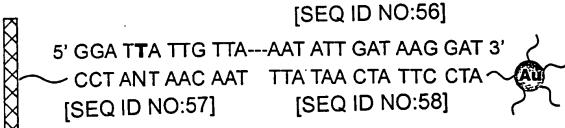












N = A (complementary), G,C,T (mismatched)

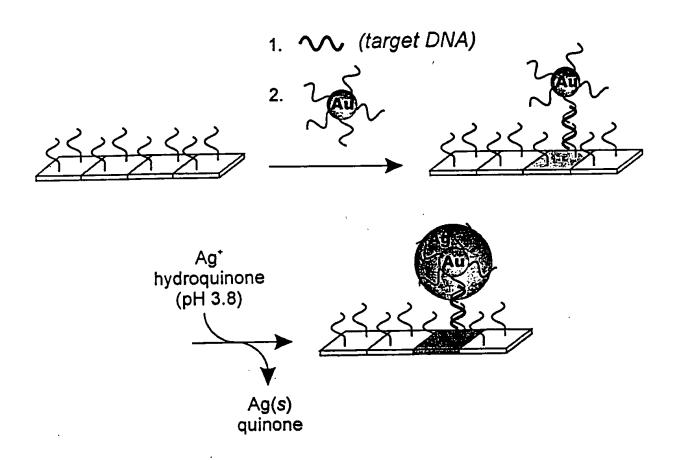


Figure 32

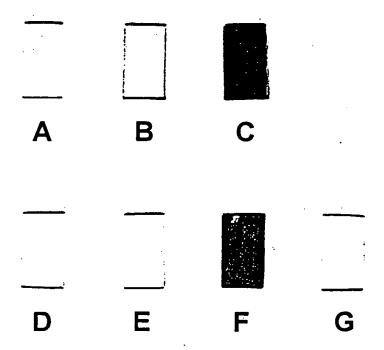


Figure 33

j

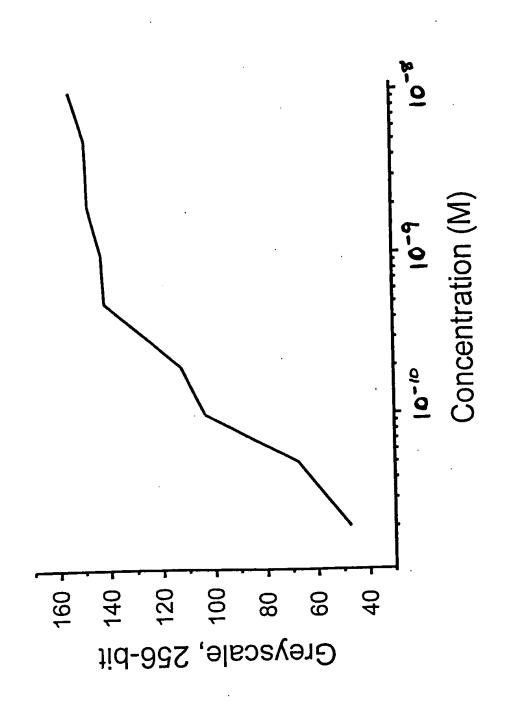
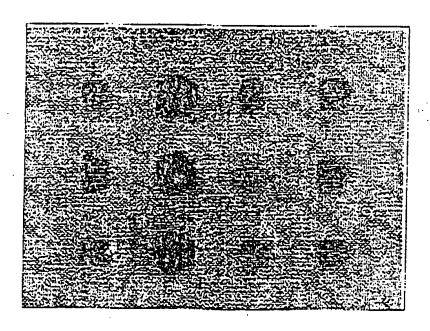


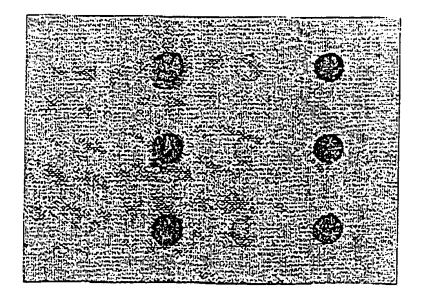
Figure 34

Figure 35

Fig. 364



F16.36B



C 🛕 T G

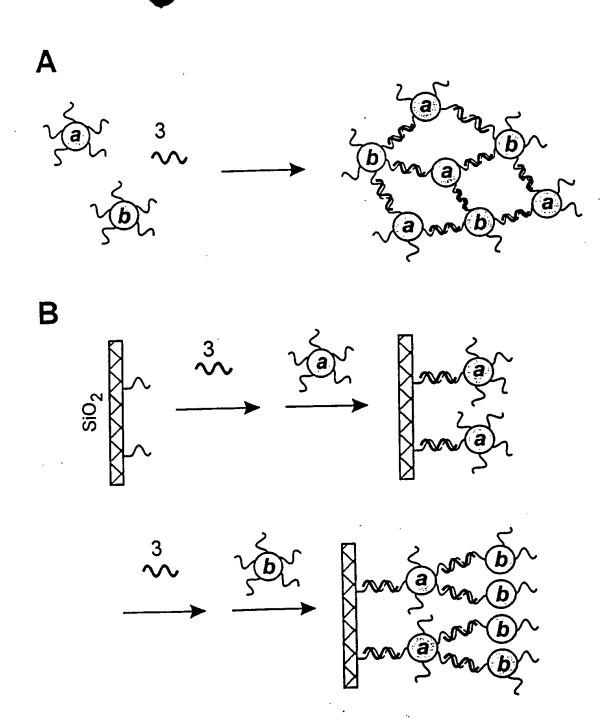


Figure 37

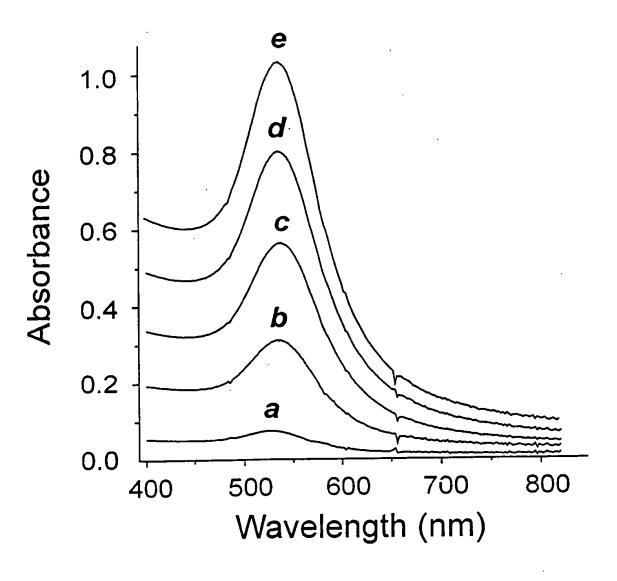


Figure 38A

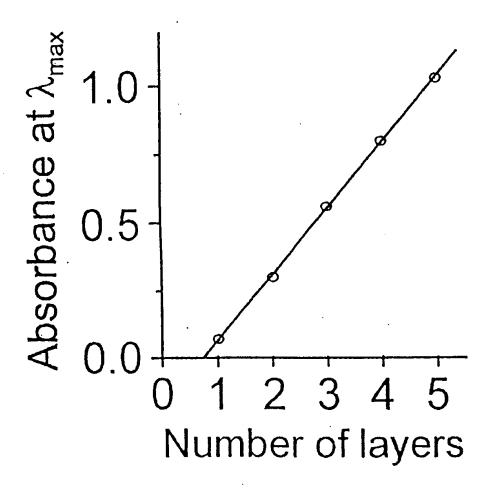
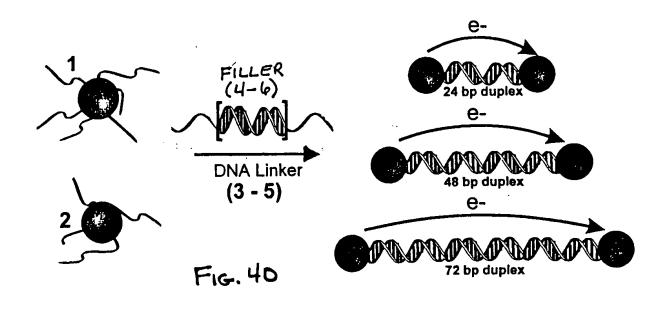


Figure 38B

Figure 39



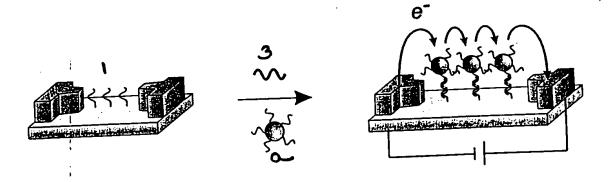


FIG. 41